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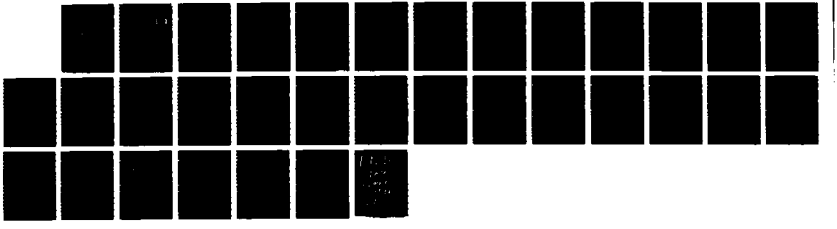

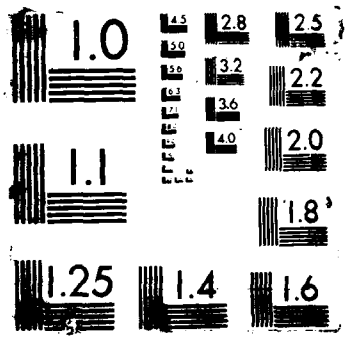
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RF Project 765335/718239
Annual Report

MECHANISMS OF CHEMICAL MODULATION AND TOXICITY OF THE IMMUNE SYSTEM

Melinda J. Tarr
Richard G. Olsen
Department of Veterinary Pathobiology

For the Period
April 15, 1987 - April 14, 1988

U.S. AIR FORCE
Air Force Office of Scientific Research/Pkd
Bolling AFB, D.C. 20332

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The results of experiments performed during this reporting period assess possible mechanisms involved in immunoregulation by 1,1-Dimethylhydrazine (UDMH). Treatment of mice with UDMH suppresses activity and production of interleukin-1 (IL1) by partially blocking IL1 receptor expression. Interleukin-2 (IL2) activity and production was also suppressed by UDMH, although the basal level of IL2 receptor was not affected. UDMH may partially reverse the immunosuppressive effects of Corynebacterium parvum in mice by interfering with PGE2 production, but not hydrogen peroxide. Preliminary results show UDMH may affect murine splenic T-lymphocyte populations defined by helper/inducer (L3T4) and suppressor/cytotoxic (LYT-2) monoclonal antibodies. Antigen presenting B-cells and macrophage also show variable Ia antigen expression upon exposure to UDMH.

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I. Research Objectives

The following is a list of original or modified specific aims for which work has been done or is ongoing since the last annual technical report (June 1987).

A. Further characterize the *in vivo* and *in vitro* effects of UDMH on interleukin 1 (IL1) and interleukin 2 (IL2) synthesis and activity.

The *in vivo* effects of UDMH on IL2 synthesis have been evaluated since the last annual report. Results of all other experiments for this specific aim were reported previously. One manuscript has been submitted for publication and another will soon be ready for submission.

B. Evaluate the effects of UDMH on IL2 receptor expression and IL2 adsorption.

The IL2 adsorption experiments have been completed and the receptor expression experiments are in progress.

C. Investigate the effects of UDMH on *in vivo Corynebacterium parvum (C. parvum)*-induced suppression of lymphocyte blast transformation (LBT) and *in vitro* hydrogen peroxide- (H_2O_2) induced LBT suppression.

A few more experiments were done with the effects of H_2O_2-induced immunosuppression. No more was done with the *C. parvum* experiments and a manuscript is in preparation summarizing these experiments.

D. Evaluate the *in vitro* effects of UDMH on distribution and expression of T-lymphocyte subset antigens and Ia antigens using flow cytometry.

This specific aim has been the main focus of our efforts this past year. Both *in vitro* and *in vivo* UDMH-exposed splenocytes have been evaluated.

E. Determine the *in vitro* immunomodulatory effects of UDMH at different exposure times during the immune response.

No experiments have been done or are currently planned for this objective (see "Status of Research").

F. Evaluate the effects of UDMH on suppressor T-lymphocyte function.

No additional work has been done during the past year but this objective will receive high priority during the coming year.

G. Determine the effects of UDMH on cell membrane calcium flux and intracellular calcium ionization (new specific aim).

II. Status of Research

Following is a detailed account of the rationale, methods, results, and significance for each specific aim.

A. Effects of UDMH on IL1 and IL2 production and activity.

1. IL1

a. Rationale

The macrophage-derived protein, interleukin 1 (IL1), appears to play a critical role in immunological responses. *In vitro* IL1 has been found responsible for regulating T-cell proliferation by inducing interleukin 2 (IL2) production by T-lymphocytes, and enhancing B-cell differentiation and antibody secretion. Fever induction, stimulation of acute-phase protein synthesis and induction of neutrophilia are some *in vivo* properties of IL1. Agents altering the ability of macrophages to synthesize and secrete IL1 would undoubtedly modulate the immune system due to multiple IL1 functions. Since many of our previous experiments indicate that the macrophage is a target cell for UDMH-induced immunomodulation, the effects of UDMH on IL1 production and activity were evaluated.

b. Methods

The cell source of IL1 was an adherent macrophage cell line, $P388_{D1}$. Cells were grown to confluency, then cultured with phorbol myristate acetate (PMA) for 5 hrs, then washed and further cultured in the presence of fresh medium for 24 hrs. The supernatant was harvested and assayed for IL1 activity using thymocytes from C3H/HeJ mice. The thymocytes were cultured with phytohemagglutinin (PHA)

and IL1 supernatant for 24, 48, or 72 hrs, and then evaluated for proliferative activity by measurement of tritiated thymidine uptake.

c. Results

The results of the *in vitro* experiments were reported in the last annual report. In summary, UDMH slightly suppresses the production of IL1 by PMA-stimulated $P388_{D1}$ cells, and markedly suppresses the activity of IL1 on thymocytes. Experiments were then done to try to determine the mechanism or site of action of UDMH-induced suppression of IL1 activity. Three possibilities were tested: 1) inactivation of the IL1 molecule by UDMH; 2) interference of IL1 receptor generation by UDMH; and 3) interference of the absorption of the IL1 molecule to the IL1 receptor by UDMH.

i) Effects of UDMH on IL1 molecule: As reported in the last progress report, incubation of UDMH with IL1-containing supernatant under various conditions did <u>not</u> affect the IL1 activity of that supernatant.

ii) Effects of UDMH on thymocyte receptor generation: UDMH was added to thymocytes alone, with PHA and IL1 for 24 hrs (to generate receptors) then the supernatant was tested for IL1 activity on fresh thymocytes. As shown in Table 1, the control supernatant (without UDMH) had fairly low IL1 activity, indicating that IL1 had been absorbed onto the generated IL1 receptors on the initial thymocytes. However, when UDMH was added during the receptor generation time, the IL1 activity of the supernatant remained high, indicating that the IL1 receptors had not been generated on the initial thymocytes.

iii) Effects of UDMH on receptor adsorption of IL1 molecule: For these experiments, thymocytes were incubated with IL1 and PHA for 24 hrs to generate receptors, then UDMH was added for another 24 hrs, and then the supernatant was tested for IL1 activity on fresh thymocytes. As shown in Table 2, the presence of UDMH did not affect the IL1 activity of the

supernatant, indicating that it did not interfere with adsorption of the IL1 to the generated receptors.

The conclusion to be drawn from these experiments is that UDMH inhibits IL1 activity at least in part by blocking IL1 receptor generation or expression on the thymocyte cell surface.

d. Significance: See section B-4.

2. IL2

a. Rationale

Interleukin 2 (IL2) is a lymphokine produced by the helper T-cell population following antigen or mitogen stimulation. *In vitro*, IL2 has been shown to increase antibody synthesis, promote cytotoxic T-cell proliferation and allow the maintenance of long-term T-cell culture lines. *In vivo* administration of IL2 in mice produced accelerated allograft rejection and regression of established tumors. Based on its immunoenhancing properties, IL2 is now being examined as a possible immunotherapeutic agent.

Although the macrophage appears to be the primary target cell of UDMH-associated immunomodulation, hence IL1 would be more likely altered, it is important to evaluate the effects of UDMH on T-cell functions such as IL2 production and activity, since there may be multiple mechanisms of action of UDMH. Also, the assays for IL2 production and activity are currently in use in our laboratory for other projects, so it was easy to "plug" UDMH into the system.

b. Methods

Two sources of IL2 were used. One was splenocytes from normal or UDMH-treated mice which were cultured with 1.6 μg/ml concanavalin A (to stimulate IL2 production) for 24 hrs; the supernatant was then harvested for IL2 activity (see below). The

second source of IL2 was a murine helper T-cell line, EL-4, cultured in the presence of phorbol myristate acetate (PMA) with or without UDMH; the supernatant was harvested after 24 hrs and assayed for IL2 activity. IL2 activity was assayed using a murine T-cell line, CTLL-20, which is dependent on IL2 for proliferation following stimulation by phytohemaglutinin (PHA). The culture medium contained PHA and 5% IL2-containing supernatant (test as well as a standard control). After 24 hrs of incubation, CTLL-20 proliferation was evaluated by measurement of tritiated thymidine incorporation.

c. Results

A summary of the results presented in the last annual report indicate that *in vitro* UDMH exposure does not significantly suppress IL2 production by splenocytes, and slightly suppresses EL-4 production of IL2. However, UDMH does interfere with IL2 activity on CTLL-20 cells.

In vivo exposure of mice to UDMH (0, 10, 25, 50, 75 or 100 mg/kg daily for 7 days) resulted in significant suppression of splenocyte IL2 production at 50 and 100 mg/kg (Table 3). The 100 mg/kg dose was toxic, and most of the mice in that dose group died.

d. Significance: See section B-4.

B. Evaluate the Effects of UDMH on IL2 Adsorption and IL2 Receptor Expression

1. Rationale

Antigen- or mitogen-stimulated T-cells express IL2 receptors, which allows IL2 binding and subsequent proliferation. Both the IL2 molecule and its receptors have been characterized and monoclonal anti-murine IL2 receptor antibodies have been developed. These experiments were designed to determine if UDMH exerts its suppressive effects on IL2 activity by inhibiting IL2 receptor expression or IL2 binding.

2. Methods

   a. IL2 adsorption: CTLL-20 cells were incubated with IL2 in the presence or absence of UDMH for 24 hrs (which results in inactivation of UDMH); then the supernatant was assayed for IL2 activity.

   b. IL2 receptor expression: IL2 receptor expression in both *in vivo* and *in vitro* UDMH treated mouse splenocytes will be determined by the use of monoclonal anti-IL2 receptor antibody and flow cytometry. Splenocyte cultures will be stimulated with mitogen to obtain IL2 receptor expression. After 48 hrs incubation, cells will be stained by indirect immunofluorescence and fixed in 1% paraformaldehyde. These cell preparations will then be assayed for receptor expression on EPICS 753 flow cytometer.

3. Results

   a. IL2 adsorption: UDMH does not interfere with IL2 adsorption on the initial CTLL-20 cells as both control and UDMH-treated supernatants had similar activity on the fresh (second) CTLL-20 culture (Table 4).

   b. IL2 receptor expression: Preliminary results of IL2 receptor expression on *in vitro* and *in vivo* UDMH-treated cell populations without mitogen stimulation (resting state) shows that UDMH does not affect any basal IL2 receptor expression (Table 5). These basal level values were not above the control values for non-specific binding. Experiments to evaluate the effects of UDMH on IL2 receptor expression by stimulated cells are currently being done.

4. Summary and significance

   These experiments indicate that UDMH suppresses IL1 activity by interfering with IL1 receptor expression on lymphocytes (thymocytes), and that it interferes with IL2 activity by an as yet undetermined mechanism. The *in vivo* experiments showed mild suppressive effects of UDMH treatment on the ability of treated mice to produce IL2. As

mentioned in the previous progress report, the significance of the *in vitro* experimental results is uncertain as it is difficult to extrapolate from such specific artificial *in vitro* experiments to the whole animal; however, suppressed IL2 responses are seen in animals and human patients with some autoimmune diseases of which aberrant enhancement of the immune response against the host is a feature. Also, if it turns out that IL2 receptor expression is suppressed by UDMH similar to IL1 receptor expression, this may reflect a common pathway of UDMH-induced immunomodulation -- interference with cell-to-cell communication during the immune response.

C. Effects of UDMH on *C. parvum*-induced Suppression of Lymphocyte Blast Transformation (LBT) and H_2O_2-induced LBT Suppression

1. Rationale

It is well established that mice injected with macrophage activating agents such as *C. parvum* exhibit suppression of certain immune functions, including the LBT response. Since it has been shown that UDMH suppresses the chemiluminescence response of *C. parvum*-activated macrophages, it was of interest to determine if UDMH could reverse the suppressive effects of *C. parvum* on the LBT, as well as IL2 production. *In vitro* experiments were also done in which macrophages from *C. parvum*-treated mice were exposed to UDMH, then assayed for their suppressive effect when added to LBT cultures.

One mechanism of suppression of the LBT by activated macrophages is purported to be membrane lipid peroxidation induced by H_2O_2 (produced by activated macrophages). To determine if inactivation of H_2O_2 is the mechanism by which UDMH reverses the suppressive effects of *C. parvum*-activated macrophages, UDMH and H_2O_2 were added to mitogen-stimulated LBT cultures.

2. Methods

H_2O_2-induced LBT suppression: In addition to the experiments described in the last progress report, other experiments were done in

which splenocytes were preincubated with H_2O_2, then washed and cultured with UDMH in the LBT assay.

3. Results

   a. Effects of UDMH on *C. parvum*-induced LBT suppression: As previously reported, *in vivo* UDMH treatment partially reversed the marked suppression of lymphocyte blast transformation (LBT) and IL2 production in *C. parvum*-treated mice. In vitro experiments to try to determine which cell type (adherent, macrophage-enriched or non-adherent, T-cell enriched) UDMH was affecting to cause this reversal were performed, but the results were inconclusive. A manuscript detailing the *in vivo* experiments is in preparation.

   b. Effects of UDMH or H_2O_2-induced LBT suppression. These experiments were done to determine if UDMH could reverse H_2O_2-induced damage which results in LBT suppression, or protect against such damage. The results in Tables 6 and 7 show that UDMH did not reverse or enhance the suppression induced by H_2O_2 preincubation. Tables 8 and 9 show that preincubation with UDMH did not alter subsequent H_2O_2-induced suppression.

4. Significance

   These experiments indicate that UDMH does partially reverse the immunosuppressive effects of *C. parvum* in mice. Other researchers have shown that this effect is due to both PGE_2 and H_2O_2 produced by activated macrophages (Metzger *et al., J. Immunol.* 124:983, 1980). Although not directly proven, our experiments from this and previous years would suggest that UDMH may reverse the *C. parvum*-induced suppression (as well as exerting its other immunoenhancing properties) at least partly by interfering with PGE_2 production (Tarr *et al., Toxicology in vitro*, in press, 1988) and <u>not</u> by interfering with H_2O_2-induced immunosuppression.

D. Re-evaluation of *in vitro* and *in vivo* Effects of UDMH on T-lymphocyte Subset Antigen and Ia Antigen Distribution and Expression Using Flow Cytometry.

1. Rationale

a. T-lymphocyte antigens: T-lymphocytes bear surface antigens which correlate with their function. Murine lymphocytes can be divided into helper/inducer cells expressing the L3T4 surface antigens and suppressor/cytotoxic cells bearing Lyt-2 surface antigens. Autoimmune or immunodeficient diseases have been characterized by alterations in the ratio or absolute number of these lymphocyte populations. Flow cytometric analysis of expression of these cell surface antigens upon exposure to UDMH both *in vivo* and *in vitro* are being examined.

b. Ia antigens: The Ia antigen is a cell surface marker and product of an immune response gene. It is present on B cells and macrophages (antigen presenting cells) and suppressor T-lymphocytes. Other T-lymphocytes interact with the Ia antigen via a receptor molecule to allow optimum immune response. Alteration of or interference with Ia antigen expression may indicate the immunomodulatory effects of UDMH. Flow cytometric analysis of Ia antigen expression on B cells and macrophage of *in vitro* and *in vivo* UDMH exposed murine spleen cells are being examined.

2. Methods

a. Lymphocyte antigens: Lymphocytes were isolated from the spleen, mesenteric lymph nodes, and thymus of normal and UDMH-treated mice. Adherent cells were removed and nonadherent cell suspensions were incubated with UDMH (cells from normal mice only). Direct immunofluorescence staining was then performed using fluorescein and phycoerythrin-conjugated monoclonal anti-L3T4 or Lyt-2 or Thy 1.2 (a general T-cell marker) antibodies. These cells were then evaluated for percent cells

expressing antigens and density of Ag per cell using flow cytometry.

b. Ia antigen: Spleen cells from normal or UDMH-treated mice were enriched for adherent populations of B-cells and macrophages. Cells were incubated with UDMH (normal cells only) and FITC-conjugated monoclonal anti-Ia antibody. They were evaluated as described above using flow cytometry.

3. Results

a. Lymphocyte antigens: Preliminary results of lymphocyte surface antigen expression in UDMH-treated murine cell populations are reported in Tables 10, 11 and 12. These values are representative of one *in vivo* experiment and fall within the range of normal lymphocyte populations determined by monoclonal antibodies (Ledbetter *et al., J. Exp. Med.* 152:280-295 [1980] and Dialynas *et al., Immunol. Rev.* 74:28-56 [1983]). In our assay system, the splenic lymphocyte populations appear to be more affected by UDMH than either mesenteric lymph node or thymic lymphocyte populations. UDMH appears to exert a dose-dependent suppression of Thy 1.2 and a variable response of Lyt-2 and L3T4 defined splenic lymphocytes. The overall surface antigen density as expressed by mean channel fluorescence (MCF) does not seem to be affected by UDMH.

b. Ia antigen: Table 13 represents the UDMH-treated adherent B-cells and macrophage expressing surface Ia antigen as determined by monoclonal antibody and flow cytometric analysis. A slight increase in the percent of Ia antigen expressing cells is seen with increasing doses of UDMH. This does not appear to be due to an increase in antigen density as determined by the mean channel fluorescence.

4. Significance

a. The results presented in Tables 10, 11, 12 and 13 are only preliminary. The importance of UDMH on lymphocyte and macrophage antigen expression cannot be fairly represented by

one experiment, but an interesting correlation of present and previous work can be seen. We previously reported immunomodulation of mixed lymphocyte reaction and Ts cell activity with UDMH. The changes represented in the antigen presenting cells by expression of Ia antigen and Ts cell function could account for this immunoregulatory activity. Additional experiments are currently in progress to determine the validity of the results presented here.

E. Determine the *in vitro* Immunomodulatory Effects of UDMH at Different Exposure Times During the Immune Response

1. Rationale

In previous experiments in which the *in vitro* effects of UDMH on the lymphocyte blast transformation (LBT) assay and mixed lymphocyte response (MLR) assay were evaluated, UDMH was always added at the beginning of the culture period. Since UDMH becomes degraded in aqueous solutions of pH 7.0 at 30°C, it would not be in its active or native form during later stages of the immune response, particularly during lymphoproliferation (which begins approximately 24 hrs or more after exposure to the mitogen or allogeneic cell). In light of our finding that UDMH inhibits proliferation of CTLL-20 cells in the presence of IL2, it was of interest to determine what effects active UDMH had upon LBT and MLR when added at various times after culture initiation.

2. Summary of Results and Significance

As noted in the last report, UDMH caused either no effect or a suppressive effect on both the MLR and LBT when added 24 or 48 hrs after the beginning of the assay compared to when it was added at the beginning. When additional suppression occurred, it was usually associated with significant cytotoxicity and at higher concentrations. It was concluded that UDMH is more toxic for proliferating lymphocytes. This may indeed have consequences in UDMH exposed animals, if significant exposure happened to occur during various stages of the immune response in which lymphoproliferation was

occurring. However, this premise would be impossible to test, as lymphocyte populations cannot be separated or enumerated *in vivo*.

F. Evaluate the Effects of UDMH on Suppressor T-lymphocyte Functions

1. Rationale

The current scope of our research is based on the premise that UDMH interferes with immunoregulation. There are several known or theoretical mechanisms of immunoregulation, some of which are described in the introduction to the proposal. To date, most of our efforts have centered around the effects of UDMH on immunoregulation by macrophages; but another major immunoregulatory network involves the function of suppressor T-lymphocytes. We have only briefly evaluated the effects of UDMH on suppressor T-cell (Ts) function, and we agree with the reviewers of our proposal that this is an area that should be more fully explored. The *in vitro* effects of UDMH on two non-antigen-specific suppressor T-cell systems, namely "Prostaglandin-Induced T-Cell Suppressor" (PITS)-induced suppressor T-cells, and concanavalin A (Con A)-induced suppressor T-cells, will be evaluated.

As stated in the "Research Objectives," no new work has been done on this specific aim since the last progress report. It will be a major focus of our efforts during the coming year.

G. Evaluate the *in vitro* and *in vivo* Effects of UDMH on Intracellular Calcium in Activated and Non-activated Lymphocytes. (New specific aim.)

1. Rationale

Calcium acts as an intracellular second messenger in many cellular events. Fluctuation of intracellular ionized calcium (Ca^{++}) concentrations has been shown to occur in mitogen or antigen-induced lymphoproliferation. UDMH may act to effect Ca^{++} release from intracellular pools and thus affect lymphocyte proliferation. The level of intracellular calcium can be easily measured with a cytoplasmically trapped fluorescent indicator that irreversibly binds Ca^{++}. This assay is currently in use in our laboratory for other

research projects. We propose to examine a variety of murine lymphoid cell populations exposed to UDMH to explore another possible mechanism of UDMH-induced immunomodulation.

2. Methods

Normal and UDMH-treated murine cell populations will be loaded with the fluorescent indicator, Fura-2. Fura-2 has an emission spectrum that changes with the binding of Ca++ which occurs upon the release of Ca++ from intracellular stores. This is manifest as changes in emission wave length, which is measured by a spectrofluorometer. Normal and UDMH-treated cells will be stimulated with the mitogens ConA, PHA and LPS, and Ca++ flux will be measured at various times after mitogen addition.

3. Results

These experiments are just getting underway, but early results show UDMH increases intracellular Ca(++) within a few seconds of its addition to murine splenocytes in the resting state (without mitogen).

4. Significance

The intracellular second messenger activity of Ca++ is well documented for lymphocyte proliferation (Lightman *et al., Blood* 61(3):413-422 [1983] and Rozengart, *Science* 234:161-166 [1986]). The interaction of UDMH on intracellular Ca(++) levels could help define mechanisms of UDMH-induced immunomodulation.

III. Written Publications (cumulative list)

A. Suppression of mitogen-induced blastogenesis of feline lymphocytes by *in vitro* incubation with carcinogenic nitrosamides. Tarr, M.J. and Olsen, R.G. *Immunopharmacology* 2:191-199, 1980.

B. Differential effects of hydrazine compounds on B = and T-cell immune function. Tarr, M.J. and Olsen, R.G. AGARD Conference Proceedings No. 309, Toxic Hazards in Avaiation, B3-1-7, 1981.

C. In vivo and *in vitro* effects of 1,1-dimethylhydrazine on selected immune functions. Tarr, M.J., Olsen, R.G. and Jacobs, D.L. Immunopharmacology 4:139-147, 1982.

D. Comparison of *in vitro* and *in vivo* immunotoxicology assays. Tarr, M.J., Olsen, R.G. and Jacobs, D.L. Annals N.Y. Acad. Sci. 407:469-471, 1983.

E. Species variation in susceptibility to methylnitrosourea-induced immunosuppression. Tarr, M.J. and Olsen, R.G. Env. Path. Toxicol. Oncol. 6:261-269, 1985.

F. Chemical alteration of host susceptibility to viral infection. Tarr, M.J. In: Comparative Pathobiology of Viral Diseases, Olsen, R.G., Krakowka, S. and Blakeslee, J.R., Jr., ed. pp. 47-55, 1985.

G. Enhancement of murine mixed lymphocyte response by 1,1-dimethylhydrazine: Characterization and possible mechanism. Tarr, M.J., McKown, B.J., and Olsen, R.G. Cancer Detect. Prevent., 1988.

H. In vitro modulation of macrophage functions by 1,1-dimethylhydrazine. Tarr, M.J., Olsen, R.G., Bowen, B.L., and Fertel, R.H. In press, Toxicology in Vitro, 1988.

I. Effects of 1,1-dimethylhydrazine on immunosuppression in mice treated with *Corynebacterium parvum*. Bauer, R.M., Tarr, M.J., and Olsen, R.G., in preparation.

J. Effects of 1,1-dimethylhydrazine on macrophage oxygen metabolite production and immunosuppressive activity. Frazier, D.E., Tarr, M.J., and Olsen, R.G. In preparation.

K. Effects of 1,1-dimethylhydrazine on lymphoproliferation and interleukin 2 regulatory function. To be submitted to Arch. Environ. Contam. Toxicol.

L. Effects of 1,1-dimethylhydrazine on interleukin 1 production and activity. Bauer, R.M., Tarr, M.J. and Olsen, R.G. In preparation.

IV. Professional Personnel Associated with Research Effort

A. Melinda J. Tarr, C.V.M., Ph.D., Principal Investigator, Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio 43210.

B. Richard G. Olsen, Ph.D., Co-Principal Investigator, Departments of Veterinary Pathobiology, Microbiology (College of Biological Sciences), and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210.

C. Donald E. Frazier, Jr., B.S., M.S. (graduate research associate)

Mr. Frazier earned his Master of Science degree in summer 1987, and is now working towards a Ph.D. He is fully supported by this grant.

D. Richard M. Bauer, B.S., M.S., Ph.D.

Mr. Bauer attained his Doctor of Philosophy degree in fall 1987, and has just left the University to take a job in industry. He was partially supported by this grant.

V. Oral Presentations - April 1987 to April 1988

A. Effect of Unsymmetrical 1,1-dimethylhydrazine on Interleukin 2 Immunoregulatory Function. Bauer, R.M., Tarr, M.J. and Olsen, R.G. Presented at the 17th Conference on Toxicology, Dayton, Ohio, November 1987.

B. Immunomodulatory Effects of Hydrazine Compounds. Presented at 3rd Inter-American Society of Chemotherapy Conference, Clearwater Beach, Florida, January 1988.

Table 1. Effect of UDMH on IL1 receptor generation.

Concentration UDMH (µg/ml)	Incorporation of ^{3}H-TdR (cpm)	p
0	21619. ± 2049	-
10	89266.75 ± 1237	<.001
25	90265 ± 1704	<.001
50	93210 ± 1704	<.001
75	89542.25 ± 1626	<.001
100	91742 ± 2053	<.001

Results represent the mean ± S.D. of 4 individual experiments, each of which was assayed in triplicate in the IL1 co-mitogen assay.

UDMH was added to PHA-plus IL1-stimulated thymocytes at culture initiation and the cell-free supernatant was tested for IL1 activity following 24 hours of culture.

Table 2. Effect of UDMH on the IL1 receptor.

Concentration UDMH (µg/ml)	Incorporation of ^{3}H-TdR (cpm)	P
0	17005.3 ± 2352	-
10	20667.3 ± 978	.067
25	20361.6 ± 565	.074
50	19539.6 ± 1544	.193
75	18971 ± 3673	-
100	21373.3 ± 1790	.062

Results represent the mean ± S.D. of 3 individual experiments, each of which was assayed in triplicate in the IL1 co-mitogen assay.

UDMH was added to PHA-plus IL-1-stimulated thymocytes following 24 hours of culture and the cell-free supernatant was tested for IL1 activity following another 24 hours of culture.

Table 3. Effect of UDMH administration *in vivo* on lectin-stimulated IL2 production.[a]

Dose UDMH (mg/kg)	N[b]	IL2 Activity[c] ($\bar{X}$ cpm ± S.D.)	p
0	6	25220 ± 4854	-
10	4	24753 ± 6233	.81
25	6	23245 ± 6231	.29
50	6	21886 ± 3483	.023
75	5	23558 ± 8952	.5
100	1	14404 ± 312	.001

[a] Mice were treated with and without UDMH for 7 days and splenocytes were prepared, stimulated with Con A and IL2 activity determined as described.

[b] N = number of animals.

[c] $\bar{X}$ cpm 1 S.D. ^{3}H-Tdr incorporation determined from triplicate assay of N animals.

Table 4. Effect of UDMH on the Interleukin 2 receptor.[a]

Concentration UDMH (µg/ml)	IL2 Activity[b]
0	9395 ± 2609
10	9015 ± 1466
25	9208 ± 1954
50	9433 ± 2597
75	8862 ± 2123
100	10681 ± 3262

[a] CTLL-20 cells were stimulated with IL2 in the presence and absence of UDMH. Following 24 hours of incubation, IL2 remaining in the culture medium was determined in the IL2 microassay.

[b] Mean cpm ± S.D. for 3 individual experiments.

Table 5. Basal IL-2 receptor expression in UDMH-treated cell populations.

	% Positive Cells	MCF*
0	23.65	79.92
10	15.73	77.50
25	17.32	77.75
50	15.15	79.09

*MCF = Mean channel fluorescence

Table 6. Effect of murine splenocyte preincubation with H_2O_2 in Con A stimulated LBT in presence of UDMH.

	UDMH		
H_2O_2 (μM)	(Control) 0 μg/ml	15 μg/ml	25 μg/ml
0	15680 ± 313[a]	8416 ± 800[c]	7068 ± 263[c]
10	10539 ± 905[b]	7584 ± 514[c]	3880 ± 2235[b,c]
20	2648 ± 259[b]	2625 ± 805[b]	718 ± 224[b,c]
30	605 ± 191[b]	709 ± 368[b]	338 ± 62[b]
40	146 ± 49[b]	155 ± 26[b]	93 ± 15[b]

[a] Results reported as mean CPM +/- S.D.

[b] $p \leq 3.86 \times 10^{-5}$ compared to no UDMH and no H_2O_2 (Student's T-test).

[c] $p \leq 0.029$ compared to no UDMH and corresponding H_2O_2 concentration.

Table 7. Effect of murine splenocyte preincubation with H_2O_2 in LPS stimulated LBT in presence of UDMH.

	UDMH		
H_2O_2 (μM)	(Control) 0 μg/ml	15 μg/ml	25 μg/ml
0	9165 ± 896	9048 ± 874	8491 ± 881
10	7831 ± 436[b]	8680 ± 313[c]	7451 ± 339
20	2076 ± 85[b]	3033 ± 899[b]	2039 ± 100[b]
30	319 ± 49[b]	540 ± 41[b,c]	327 ± 45[b]
40	138 ± 25[b]	188 ± 11[b]	134 ± 34[b]

[a] Results reported as mean CPM +/- S.D.

[b] $p \leq 0.037$ compared to no UDMH and no H_2O_2 (Student's T-test).

[c] $p \leq 0.019$ compared to no UDMH and corresponding to H_2O_2 concentration.

Table 8. Effects of murine splenocyte preincubation with UDMH on H_2O_2-induced suppression of Con A-stimulated LBT response.

30 Minutes UDMH mg/ml	0 µm H_2O_2[a] Control	20 µm H_2O_2	40 µm H_2O_2
0	42531 ± 1436	16287 ± 1353[b]	91 ± 24[b]
15	40085 ± 2641	15330 ± 736[b]	124 ± 22[b]
25	44951 ± 2613	14833 ± 1156[b]	77 ± 17[b]
50	46357 ± 6800	15715 ± 1156[b]	153 ± 11[b,d]
100	42108 ± 5401	12294 ± 261[b,c]	79 ± 10[b]

a Results reported as mean CPM ± S.D.
b $P \leqq 10^{-5}$ compared with no H2O2 and the corresponding UDMH concentration (T-test).
c $P \leqq .03$ compared with no UDMH and 20 µm H_2O_2.
d $P \leqq .009$ compared with no UDMH and 0 µm H_2O_2.

Table 9. Effects of murine splenocyte preincubation with UDMH on H_2O_2-induced suppression of LPS-stimulated LBT response.

30 Minutes UDMH mg/ml	0 µm H_2O_2[a] Control	20 µm H_2O_2	40 µm H_2O_2
0	14048 ± 1999	2475 ± 380[c]	77 ± 14[c]
15	11546 ± 295	2580 ± 577[c]	116 ± 36[c]
25	14010 ± 3158	2004 ± 97[c]	72 ± 22[c]
50	12729 ± 1874	2487 ± 347[c]	73 ± 17[c]
100	11696 ± 989	2158 ± 102[c]	60 ± 19[c]

a Results reported as mean CPM ± S.D.
c $P \leq .04$ compared with no H_2O_2 and no UDMH.
b $P \leq 10^{-5}$ compared with no UDMH and corresponding UDMH concentration.

Table 10. *In vivo* effects of UDMH on expression of Thyl. 2 lymphocyte surface antigen.

{UDMH} µg/ml	Thymus		Spleen		Lymph Node	
	% Positive Cells	MCF*	% Positive Cells	MCF	% Positive Cells	MCF
0	99.54	157.11	45.14	156.30	86.58	154.16
10	99.30	154.70	34.86	154.35	82.56	153.22
25	99.58	154.05	36.44	153.28	81.12	152.48
50	99.38	164.53	25.72	155.30	83.98	155.14
75	99.56	183.18	20.46	150.23	77.48	152.45

* MCF = Mean channel fluorescence

Table 11. *In vivo* effects of UDMH on expression of T-suppressor/cytotoxic lymphocyte surface antigen Lyt-2.

{UDMH} µg/ml	Thymus		Spleen		Lymph Node	
	% Positive Cells	MCF*	% Positive Cells	MCF	% Positive Cells	MCF
0	74.76	101.06	9.36	101.78	11.58	98.42
10	72.02	101.17	6.78	96.77	12.74	93.03
25	68.82	98.95	10.94	96.91	10.34	97.00
50	68.24	100.74	8.40	95.91	13.70	95.87
75	69.86	98.76	6.24	94.88	11.68	98.28

* MCF = Mean channel fluorescence

Table 12. *In vivo* effects of UDMH on expression of T-helper/induce lymphocyte surface antigen L3T4.

{UDMH} µg/ml	Thymus		Spleen		Lymph Node	
	% Positive Cells	MCF*	% Positive Cells	MCF	% Positive Cells	MCF
0	69.55	76.27	30.34	97.04	60.02	82.15
10	75.82	76.06	23.54	96.10	61.52	88.17
25	68.68	76.16	32.28	97.30	63.22	95.8?
50	61.66	74.63	18.12	91.62	65.20	99.46
75	56.56	73.09	15.04	95.81	63.42	95.4?

* MCF = Mean channel fluorescence

Table 13. Expression of I-A antigen on in vivo UDMH-treated splenic adherent cell populations.

{UDMH} µg/ml	Ia	
	% Positive Cells	MCF*
0	53.81	143.86
10	66.24	140.76
25	61.08	143.32
50	58.86	146.92
75	62.86	149.67

* MCF = Mean channel fluorescence

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